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Clinical Research Article

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Per- and Polyfluoroalkyl Substances and Hormone Levels During the Menopausal Transition

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Abstract

Context: Per- and polyfluoroalkyl substances (PFAS) are widespread chemicals that may affect sex hormones and accelerate reproductive aging in midlife women.
Objective: To examine associations between serum PFAS concentrations at baseline (1999-2000) and longitudinal serum concentrations of follicle-stimulating hormone (FSH), estradiol, testosterone, and sex hormone-binding globulin (SHBG) at baseline and through 2015-2016.
Design: Prospective cohort.
Setting: General community.
Participants: 1371 midlife women 45 to 56 years of age at baseline in the Study of Women's Health Across the Nation (SWAN).
Main Outcome Measure(s): FSH, estradiol, testosterone, SHBG.
Results: In linear mixed models fitted with log-transformed hormones and log-transformed PFAS adjusting for age, site, race/ethnicity, smoking status, menopausal status, parity, and body mass index, FSH was positively associated with linear perfluorooctanoate [n-PFOA; 3.12% (95% CI 0.37%, 5.95%) increase for a doubling in serum concentration), linear perfluorooctane sulfonate [PFOS; 2.88% (0.21%, 5.63%)], branched perfluorooctane sulfonate [2.25% (0.02%, 4.54%)], total PFOS (3.03% (0.37%, 5.76%)), and 2-(N-ethyl-perfluorooctane sulfonamido) acetate [EtFOSAA; 1.70% (0.01%,

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3.42%)]. Estradiol was inversely associated with perfluorononanoate [PFNA; –2.47% (–4.82%, –0.05%)) and n-PFOA (–2.43% (–4.97%, 0.18%)]. Significant linear trends were observed in the associations between PFOS and EtFOSAA with SHBG across parity (*P*s trend ≤ 0.01), with generally inverse associations among nulliparous women but positive associations among women with 3+ births. No significant associations were observed between PFAS and testosterone.

Conclusions: This study observed positive associations of PFOA and PFOS with FSH and inverse associations of PFNA and PFOA with estradiol in midlife women during the menopausal transition, consistent with findings that PFAS affect reproductive aging.

Key Words: PFAS, sex hormones, menopausal transition, longitudinal study, parity

Per- and polyfluoroalkyl substances (PFAS) are a large group of manmade chemicals used extensively for oil and water repellency and friction reduction and in surfactants with a wide range of industrial and consumer applications (1,2). PFAS do not break down in the environment, and some accumulate in the human body (1). Several PFAS, especially 2 legacy compounds, perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), have been detected in blood samples of almost all persons tested in the United States and other countries (3-7). PFAS are endocrine-disrupting chemicals (8) and can directly interfere with the function of estrogen and androgen receptors (9,10). PFAS have been associated with later menarche, irregular and longer menstrual cycles, and earlier menopause and thus are potential ovarian toxicants (11).

Information on PFAS and serum hormone concentrations is limited, and the evidence has been inconsistent. Epidemiologic studies have reported links between PFOS and lower estradiol (E_2) (12-15), lower progesterone (13), lower free androgen index (16), and higher follicle-stimulating hormone (FSH) levels (15) in women. Other studies found no significant associations between hormone profiles and PFOS or PFOA (17,18). Conflicting results have also been reported for other PFAS (13-17). Most previous studies were cross-sectional with hormone levels measured at a single time point in highly selected samples of women undergoing in vitro fertilization or in women with polycystic ovarian syndrome or primary ovarian insufficiency (POI) (14-16). The lack of prospective studies in community-based samples of women has made it difficult to conclude that the observed findings were independent of reverse causality because elevated PFAS serum concentrations occur after menopause when E_2 drops and FSH increases (19).

We examined longitudinal associations between serum PFAS concentrations at baseline and serial serum concentrations of FSH, E_2, testosterone (T), and sex hormone-binding globulin (SHBG) in the Study of Women's Health Across the Nation (SWAN), a multiracial/ethnic, community-based cohort study of midlife women transitioning through menopause. We also tested whether parity modified the associations, given that ovarian toxicity of PFAS could be diminished in parous women because bleeding during parturition is an important elimination pathway for PFAS (13).

Materials and Methods

Study Population

SWAN is a community-based cohort study of the natural history of menopause designed to characterize the menopausal transition and its association with chronic diseases (http://www.swanstudy.org) (20). In 1996-1997, 3302 premenopausal women were enrolled at 7 study sites. At each site, white women and women from 1 specified minority group were recruited (black women in Boston, MA, USA; Pittsburgh, PA, USA; Southeast Michigan, USA; and Chicago, IL, USA; Hispanic women in Newark, NJ, USA; Chinese women in Oakland, CA, USA; and Japanese women in Los Angeles, CA, USA). Women were eligible if they were 42 to 52 years of age, had an intact uterus, and had had a menstrual period and were not taking hormone medications in the prior 3 months. Data and biospecimens were collected prior to 11 AM at baseline and in 15 follow-up visits approximately annually or biannually through 2016-2017. Institutional review board approval was obtained at each study site. All participants provided signed informed consent at each study visit.

The SWAN Multi-Pollutant Study (MPS) was initiated to examine reproductive health effects of multiple environmental pollutants, including PFAS, in midlife women using repository serum and urine samples from the third SWAN follow-up (MPS baseline, 1999-2000) for environmental exposure assessment (n = 2694). Women from Chicago (n = 368) and Newark (n = 278) were not eligible because urine samples were not collected at these sites. We excluded 648 women with insufficient serum or urine samples, yielding a sample of 1400 women from 4 racial/ethnic

groups (white, black, Chinese, and Japanese). For the present study, women were required to have at least 1 of the hormone outcomes measured at MPS baseline or later while not using hormone therapy or during pregnancy. We excluded all hormone measurements taken at any visits at which women were on hormone therapy. If women were missing covariates at MPS baseline, information was obtained from the prior visit. Two women who did not have information on core covariates were excluded from all analyses. Models of FSH and SHBG, measured through visit 15, had a final analytical sample of 1371 women with 10 842 and 10 841 observations. E_2 was measured through visit 13, and T, through visit 10, resulting in final analytical samples of 1361 (9920 observations) and 1331 women (7875 observations), respectively [see Supplemental Figure 1 in (21)].

Sex Hormone Assessment

SWAN assayed E_2, T, FSH, and the binding protein SHBG from fasting serum samples obtained in the early follicular phase (days 2-5). Two attempts were made to obtain an early follicular sample. If a follicular phase sample could not be collected, a random fasting blood sample was obtained within 90 days of the anniversary date of the baseline visit. For women who were postmenopausal, a blood sample was drawn at their clinic visit, scheduled to be on the anniversary of their previous visit. FSH, T, and SHBG assays were conducted in singlicate and E_2 assays in duplicate using the automated Ciba Corning Diagnostics ACS-180 analyzer (Bayer Diagnostics Corp., Norwood, MA, USA). Serum E_2 concentrations were measured with a modified, offline ACS-180 immunoassay, with a lower limit of detection (LLD) of 1 pg/mL. FSH and SHBG were measured with a 2-site chemiluminometric immunoassay, with LLDs of 1.1 IU/L for FSH and 2 nM for SHBG. T concentrations were determined by the modified rabbit polyclonal anti-T ACS-180 immunoassay, with a LLD of 2 ng/dL. Inter- and intra-assay coefficients of variation were 10.6% and 6.4% for E_2, 12.0% and 6.0% for FSH, 10.5% and 8.5% for T, and 9.9% and 6.1% for SHBG, respectively.

PFAS Assessment

PFAS assessment was conducted at the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention (CDC). The CDC laboratory's involvement did not constitute engagement in human-subjects research. As serum concentrations of the target analytes are relatively stable over time, only MPS baseline serum was analyzed (22). Online solid phase extraction-high performance liquid chromatography-isotope dilution-tandem mass spectrometry was used to quantify 11 PFAS, including linear PFOA (n-PFOA), sum of branched PFOA isomers, perfluorononanoate (PFNA), perfluorodecanoate, perfluoroundecanoate, perfluorododecanoate, perfluorohexane sulfonate (PFHxS), linear PFOS (n-PFOS), sum of perfluoromethylheptane sulfonate isomers (Sm-PFOS), and 2 PFOS precursors (23), namely 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA) and 2-(N-ethyl-perfluorooctane sulfonamido) acetate (EtFOSAA) (24). Total PFOS was computed as the sum of n-PFOS and Sm-PFOS. We did not include sum of branched PFOA isomers, perfluorodecanoate, perfluoroundecanoate, and perfluorododecanoate in data analyses due to low detection rates (<50%). Detection rates of included PFAS were >97%. Concentrations below the limit of detection (LOD) were substituted with LOD/$\sqrt{2}$. The LOD for all analytes was 0.1 ng/mL. Comprehensive quality assurance/quality control procedures were conducted. The coefficient of variation of low- and high-concentration quality controls ranged from 6% to 12%, depending on the analyte.

Covariates

Annual visits included an in-person interview, self-administered questionnaires, and measurements of weight and height. Sociodemographic variables included age, race/ethnicity, study site, and education. Race/ethnicity was classified into self-identified black, Chinese, Japanese, or white. Education was categorized as some high school, high school degree, some college, college degree, or postcollege. Health-related variables at the SWAN MPS baseline included smoking status (never, former, or current smoker), secondhand smoking, physical activity, and parity (nulliparous, 1-2 births, or 3+ births). Total person-hours of secondhand smoke exposure was calculated from 7 questions about exposure during a typical week at home, work, and in other public environments and dichotomized as any exposure *vs* none (25). Physical activity was measured using a modified Baecke questionnaire, which included 3 indices (sports and exercise activity, nonsports leisure activity, and household and childcare activity), with a minimum possible score of 3 and maximum of 15 (26). Time-varying body mass index (BMI) and menopausal status were considered. For analyzing the mean effects of PFAS on hormone levels, menopausal status was categorized into 2 groups [pre- and early perimenopausal and late peri- and postmenopausal (natural and surgical)]. Race/ethnicity and geographical location were strongly associated with PFAS concentrations (3), but only certain study sites included black, Chinese, and Japanese women; therefore, site and race/ethnicity were combined into 1 categorical variable (black women in Boston,

white women in Boston, black women in Pittsburgh, white women in Pittsburgh, black women in Southeast Michigan, white women in Southeast Michigan, Chinese women in Oakland, white women in Oakland, Japanese women in Los Angeles, and white women in Los Angeles).

Statistical Analysis

We examined the distributions of population characteristics at the MPS baseline. We calculated means and SDs of continuous covariates, medians, and interquartile ranges for PFAS and hormones due to right skewness and frequencies for categorical covariates. To assess the association between PFAS concentrations and hormone levels across the menopausal transition, we ran linear mixed effects models with a random intercept and unstructured covariance. All hormone outcomes were natural-log transformed, and all PFAS were log-2 transformed. A log-base 2 transformation was applied to PFAS so that coefficients could be interpreted as percentage change of each hormone associated with a doubling in PFAS concentrations. We also categorized PFAS concentrations into tertiles. Each hormone was regressed on each PFAS individually. We selected potential confounders a priori including age (time-varying), menopausal status (time-varying), site/race-ethnicity, smoking status, parity, and BMI (time-varying). We chose BMI as time-varying because hormone levels highly depend on BMI during the menopausal transition (19). We also evaluated the associations without adjustment for BMI to examine the influence of BMI on the PFAS-hormone associations because BMI and body weight may be influenced by PFAS exposure (27). To evaluate the robustness of the associations, we conducted 2 sensitivity analyses. First, we ran models using baseline BMI (time-constant) instead of time-varying BMI. Second, we also evaluated the associations by time-varying BMI categories by adding an interaction between PFAS and BMI categories (<25, 25-29.9, ≥30 kg/m^2). As sensitivity analyses, we additionally adjusted for education, physical activity, or secondhand smoking status.

To determine if the relationship between PFAS and hormone levels differed by parity and, in particular, if stronger associations were seen within nulliparous women, we ran all adjusted models with an interaction between PFAS and parity (nulliparous, 1-2 births, 3+ births). We calculated estimates for the PFAS/hormone associations for each parity category from the interaction models. For all models, coefficients were back-transformed using exponentiation and percent changes and 95% CIs were calculated. Statistical significance was defined at $\alpha < 0.05$ and borderline significance at $\alpha < 0.1$.

To evaluate the associations of hormones with PFAS as mixtures as an exploratory analysis, we conducted a 2-stage modeling approach to account for correlations in both dependent and independent variables (28). Stage 1 accounted for correlations in repeatedly measured outcomes within each participant. Hormone levels were regressed on time-varying covariates (age, menopausal status, BMI) in linear mixed effects models, and participant-specific hormone levels (random intercepts) were estimated. Stage 2 accounted for correlations among exposure variables, using an adaptive elastic-net method (29). A linear combination of standardized PFAS variables (exposures) were regressed on participant-specific hormone estimates from Stage 1 with adjustment for site/race-ethnicity, smoking status, and parity (time-constant covariates). Details of adaptive elastic-net and modeling procedures are provided in the Supplemental Material in (21).

All statistical analyses were conducted in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) except the mixture analysis, which was performed in R using the package gcdnet (version 1.0.5) for adaptive elastic-net.

Results

At the MPS baseline, mean age was 49.5 years (SD = 2.6) and the mean BMI was 28.0 kg/m^2 (SD = 7.4) (Table 1). The proportions of each race/ethnic group were 49.8% for white, 22.2% for black, 15.1% for Japanese, and 12.9% for Chinese. Median concentrations of hormones measured at MPS baseline were 30.0 pg/mL for E_2; 34.1 IU/L for FSH; 33.6 ng/dL for T; and 38.7 nM for SHBG. Median concentrations of PFAS were 4.1 ng/mL for n-PFOA, 0.6 ng/mL for PFNA, 1.5 ng/mL for PFHxS, 17.5 ng/mL for n-PFOS, 7.3 ng/mL for Sm-PFOS, 24.9 ng/mL for total PFOS, 1.5 ng/mL for MeFOSAA, and 1.2 ng/mL for EtFOSAA. [More details of PFAS statistics are presented in Supplemental Table 1 in (21)]

After adjustment for age, site/race-ethnicity, smoking status, menopausal status, parity, and BMI, FSH was positively associated with n-PFOA [3.12% (95% CI 0.37%, 5.95%) increase in risk for a doubling in serum concentration], n-PFOS [2.88% (95% CI 0.21%, 5.63%)], Sm-PFOS [2.25% (95% CI 0.02%, 4.53%)], total PFOS [3.03% (95% CI 0.37%, 5.76%)], and EtFOSAA [1.70% (95% CI 0.01%, 3.42%)] (Table 2). Percentage changes in FSH, comparing the top with the bottom tertiles, were 6.74% (95% CI 0.88%, 12.94%) for n-PFOA (P for trend = 0.02), 4.87% (95% CI −0.67%, 10.72%) for n-PFOS (*P* for trend = 0.09), and 5.64% (95% CI −0.17%, 11.78%) for EtFOSAA (*P* for trend = 0.05). When BMI was not included in the adjustment, associations with FSH were not significant [see Supplemental Table 2 in (21)].

E_2 was significantly or borderline significantly inversely associated with PFNA [−2.47% (95% CI −4.82%, −0.05%)] and n-PFOA [−2.43% (95% CI −4.97%, 0.18%)].

Table 1. Baseline (1999-2000) characteristics of the MPS study population, the Study of Women's Health Across the Nation (N = 1371).

Characteristics	Statistics
PFAS (ng/mL), median (Q1, Q3)	
n-PFOA	4.1 (2.9, 5.8)
PFNA	0.6 (0.4, 0.8)
PFHxS	1.5 (1.0, 2.4)
n-PFOS	17.5 (12.5, 24.9)
Sm-PFOS	7.3 (4.7, 11.0)
Total PFOS	24.9 (17.6, 35.8)
MeFOSAA	1.5 (0.9, 2.3)
EtFOSAA	1.2 (0.7, 2.2)
Sex hormone, median (Q1, Q3)[a]	
FSH, IU/L	34.1 (14.8, 84.8)
E_2, pg/mL	30.0 (17.5, 67.3)
Testosterone, ng/dL	33.6 (23.8, 46.9)
SHBG, nM	38.7 (25.4, 54.3)
Covariate, mean ± SD or n (%)	
Age, year	49.5 ± 2.6
Body mass index, kg/m^2	28.0 ± 7.4
Site	
Southeast Michigan, USA	254 (18.5)
Boston, MA, USA	231 (16.9)
Oakland, CA, USA	303 (22.1)
Los Angeles, CA, USA	356 (26.0)
Pittsburgh, PA, USA	227 (16.6)
Race/ethnicity	
White	683 (49.8)
Black	304 (22.2)
Chinese	177 (12.9)
Japanese	207 (15.1)
Education	
Some high school	41 (3.0)
High school	208 (15.2)
Some college	439 (32.2)
College	334 (24.5)
Postcollege	342 (25.1)
Smoking status	
Never	864 (63.0)
Former	364 (26.6)
Current	143 (10.4)
Secondhand smoking exposure	
None	816 (59.5)
Any	555 (40.5)
Parity	
Nulliparous	265 (19.3)
1-2	713 (52.0)
≥3	393 (28.7)
Menopausal status[b]	
Pre or early peri	924 (67.4)
Late peri or post (natural and surgical)	447 (32.6)

Abbreviations: E_2, estradiol; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate FSH, follicle-stimulating hormone; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; n-PFOA, linear perfluorooctanoate; n-PFOS, linear perfluorooctane sulfonate; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; SHBG, sex hormone-binding globulin; Sm-PFOS, sum of perfluoromethylheptane sulfonate isomers.

[a]Measure taken from first visit not using hormone therapy.

[b]Menopausal status from first visit not using hormone therapy.

A significant association of E_2 was also found with tertiles of PFNA [– 6.59% (95% CI –11.6%, –1.30%) comparing the top *vs* the bottom tertiles; *P* for trend = 0.02]. No statistically significant association was observed between PFAS and SHBG, although SHBG was significantly inversely associated with Sm-PFOS [– 4.31% (95% CI –6.84%, –1.72%)] and total PFOS [–3.45% (95% CI –6.48%, –0.32%)] when BMI was not included in the models [see Supplemental Table 2 in (21)]. PFAS were not significantly associated with T concentrations. No clear patterns of the association between PFAS and hormone levels were observed across BMI categories although stronger associations of PFNA with E_2 [–4.47% (95% CI –7.83%, –0.99%)] and T [4.35% (95% CI 0.66%, 8.17%)] were observed in women with BMI < 25 kg/m^2 whereas null associations were observed in women with BMI ≥ 30 kg/m^2 (*P*-values for linear trends < 0.1) [see Supplemental Table 3 in (21))]. The results were robust when baseline BMI was included in the adjustment instead of time-varying BMI [see Supplemental Table 4 in (21)]. The associations remained essentially unchanged with further adjustment for education, physical activity, and secondhand smoking status [see Supplemental Table 5 in (21)].

Examination of effect modification by parity revealed that associations between PFOS and EtFOSAA and SHBG differed by parity (Table 3). In the fully adjusted model, significant linear trends appeared across parity (*Ps* for trend ≤ 0.01), generally inverse associations among nulliparous women but positive associations among women with 3+ births. We did not observe significant effect modification by parity in the association between FSH and PFOA and PFOS, although a significant linear trend in effect across parity between FSH and PFHxS was observed (*P* for linear trend = 0.012).

The 2-stage modeling, combining linear mixed effects and adaptive elastic-net, that accounted for copollutant confounding and collinearity identified the following PFAS components to have nonzero coefficients: n-PFOA for FSH; n-PFOA, PFNA, and MeFOSAA for E_2; n-PFOS, PFNA, MeFOSAA, and EtFOSAA for T; and MeFOSAA for SHBG (Table 4). Only the association between n-PFOA and FSH was statistically significant [2.27% (95% CI 0.26%, 4.32%)].

Discussion

This is the first study of which we are aware to examine longitudinal associations between serum PFAS concentrations and sex hormones conducted in a community-based longitudinal cohort of midlife women. Two legacy PFAS, PFOS and PFOA, measured at baseline were associated with higher FSH concentrations, whereas PFNA and PFOA were associated with lower E_2 concentrations

Table 2. Percentage changes (95% CIs) in serum concentrations of sex hormones for a doubling in and by tertiles of PFAS concentrations

PFAS	Per doubling in PFAS concentrations	Tertiles of PFAS concentrations			*P* for trend
		T1	T2	T3	
FSH					
n-PFOA	3.12 (0.37, 5.95)**	Ref	1.88 (–3.48, 7.53)	6.74 (0.88, 12.94)**	0.02
PFNA	1.17 (–1.35, 3.75)	Ref	–2.09 (–7.01, 3.08)	1.09 (–4.50, 7.01)	0.72
PFHxS	–0.03 (–1.96, 1.94)	Ref	0.22 (–4.92, 5.63)	0.17 (–5.22, 5.87)	0.95
n-PFOS	2.88 (0.21, 5.63)**	Ref	–0.21 (–5.26, 5.11)	4.87 (–0.67, 10.72)*	0.09
Sm-PFOS	2.25 (0.02, 4.53)**	Ref	–0.08 (–5.24, 5.35)	4.54 (–1.10, 10.51)	0.11
Total PFOS	3.03 (0.37, 5.76)**	Ref	–0.67 (–5.71, 4.64)	4.07 (–1.44, 9.89)	0.16
MeFOSAA	0.13 (–2.09, 2.40)	Ref	–1.53 (–6.68, 3.91)	0.66 (–4.87, 6.50)	0.80
EtFOSAA	1.70 (0.01, 3.42)**	Ref	–0.09 (–5.27, 5.39)	5.64 (–0.17, 11.78)*	0.05
E_2					
n-PFOA	–2.43 (–4.97, 0.18)*	Ref	–5.62 (–10.44, –0.55)**	–4.02 (–9.15, 1.41)	0.16
PFNA	–2.47 (–4.82, –0.05)**	Ref	–0.70 (–5.52, 4.37)	–6.59 (–11.6, –1.30)**	0.02
PFHxS	–0.86 (–2.72, 1.03)	Ref	–1.45 (–6.35, 3.71)	–1.74 (–6.87, 3.68)	0.52
n-PFOS	–1.82 (–4.31, 0.75)	Ref	1.20 (–3.79, 6.44)	–3.05 (–8.04, 2.20)	0.26
Sm-PFOS	–1.27 (–3.38, 0.88)	Ref	–2.94 (–7.81, 2.18)	–1.75 (–6.92, 3.70)	0.53
Total PFOS	–1.72 (–4.20, 0.83)	Ref	–0.90 (–5.79, 4.25)	–1.76 (–6.83, 3.59)	0.51
MeFOSAA	0.97 (–1.21, 3.21)	Ref	4.42 (–0.88, 10.01)	–0.66 (–5.97, 4.94)	0.75
EtFOSAA	0.37 (–1.26, 2.04)	Ref	0.09 (–4.97, 5.42)	–0.34 (–5.69, 5.32)	0.90
Testosterone					
n-PFOA	0.31 (–2.68, 3.39)	Ref	–0.53 (–6.35, 5.65)	–0.87 (–6.93, 5.59)	0.79
PFNA	2.20 (–0.62, 5.11)	Ref	2.75 (–2.98, 8.82)	3.81 (–2.57, 10.62)	0.25
PFHxS	0.90 (–1.27, 3.12)	Ref	–3.99 (–9.46, 1.82)	1.08 (–4.95, 7.50)	0.74
n-PFOS	1.40 (–1.53, 4.42)	Ref	3.31 (–2.52, 9.48)	2.81 (–3.23, 9.23)	0.36
Sm-PFOS	0.52 (–1.92, 3.02)	Ref	6.08 (–0.01, 12.54)*	–0.41 (–6.37, 5.94)	0.87
Total PFOS	1.22 (–1.69, 4.21)	Ref	2.69 (–3.11, 8.85)	2.56 (–3.47, 8.97)	0.41
MeFOSAA	–1.55 (–3.99, 0.95)	Ref	–2.83 (–8.48, 3.17)	–3.32 (–9.25, 2.99)	0.30
EtFOSAA	–1.51 (–3.34, 0.35)	Ref	0.00 (–5.80, 6.16)	–5.54 (–11.32, 0.63)*	0.07
SHBG					
n-PFOA	0.86 (–2.18, 4.00)	Ref	0.72 (–5.28, 7.10)	1.25 (–5.03, 7.95)	0.71
PFNA	0.18 (–2.65, 3.09)	Ref	1.20 (–4.54, 7.29)	–1.74 (–7.88, 4.81)	0.60
PFHxS	0.39 (–1.81, 2.63)	Ref	–1.22 (–6.95, 4.86)	1.04 (–5.11, 7.58)	0.75
n-PFOS	0.98 (–1.99, 4.03)	Ref	8.82 (2.60, 15.41)***	2.88 (–3.24, 9.39)	0.33
Sm-PFOS	0.20 (–2.27, 2.73)	Ref	–0.45 (–6.26, 5.73)	0.23 (–5.88, 6.73)	0.94
Total PFOS	0.92 (–2.02, 3.95)	Ref	6.94 (0.81, 13.44)**	2.46 (–3.65, 8.96)	0.41
MeFOSAA	1.73 (–0.82, 4.34)	Ref	–0.37 (–6.25, 5.89)	4.23 (–2.22, 11.12)	0.19
EtFOSAA	1.14 (–0.76, 3.08)	Ref	0.63 (–5.28, 6.92)	4.13 (–2.32, 11.02)	0.21

Models adjusted for age (time-varying), race/ethnicity, site, smoking status, parity, menopausal status (time-varying), and BMI (time-varying).
Abbreviations: E_2, estradiol; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; FSH, follicle-stimulating hormone; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; n-PFOA, linear perfluorooctanoate; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; n-PFOS, linear perfluorooctane sulfonate; SHBG, sex hormone-binding globulin; Sm-PFOS, sum of perfluoromethylheptane sulfonate isomers. *$P < 0.10$; **$P < 0.05$; ***$P < 0.01$.

over 15 years of follow-up. The observed magnitude increase for FSH comparing the top *vs* the bottom tertiles of n-PFOA (6.74%) was roughly equivalent to the magnitudes of the association for about 2.5 years of aging, 2.5 kg/m^2 of BMI, and current smokers *vs* never smokers. For E_2, the magnitude decrease comparing the top *vs* the bottom tertiles of PFNA (6.59%) was similar to those for 4 years of aging and 7 kg/m^2 of BMI and was much larger than the smoking effect (2.2%). Significant linear trends in the associations between PFOS and EtFOSAA (PFOS precursor) and SHBG across parity were observed, suggesting that parity may play an important role in the associations between some PFAS serum concentration and sex hormones.

FSH is a well-known indirect marker of ovarian aging as FSH production and secretion is modulated by

Table 3. Percentage changes (95% CIs) in serum concentrations of sex hormones for a doubling in PFAS concentrations by parity

	Nulliparous (%)	1-2 births (%)	3+ births (%)	P for trend
FSH				
n-PFOA	2.21 (−3.05, 7.75)	2.55 (−1.20, 6.45)	4.95 (−0.06, 10.21)*	0.393
PFNA	−1.58 (−6.98, 4.14)	0.12 (−3.40, 3.75)	4.54 (0.03, 9.26)**	0.069
PFHxS	−4.62 (−8.60, −0.47)**	0.47 (−2.13, 3.14)	2.32 (−1.24, 6.00)	0.012
n-PFOS	−0.91 (−6.45, 4.96)	4.56 (0.86, 8.39)**	2.61 (−2.16, 7.61)	0.445
Sm-PFOS	0.37 (−4.04, 4.98)	2.64 (−0.43, 5.80)*	3.14 (−1.01, 7.47)	0.356
Total PFOS	−0.22 (−5.71, 5.59)	4.43 (0.74, 8.26)**	2.90 (−1.84, 7.86)	0.472
MeFOSAA	1.43 (−3.32, 6.41)	−0.91 (−3.76, 2.03)	1.41 (−2.79, 5.79)	0.998
EtFOSAA	2.86 (−0.90, 6.77)	1.30 (−0.95, 3.61)	1.68 (−1.36, 4.81)	0.666
E_2				
n-PFOA	−4.34 (−9.11, 0.68)*	−1.10 (−4.64, 2.57)	−3.03 (−7.52, 1.68)	0.838
PFNA	−3.12 (−8.32, 2.38)	−2.01 (−5.35, 1.45)	−2.77 (−6.84, 1.47)	0.984
PFHxS	1.96 (−2.15, 6.25)	−2.55 (−5.01, −0.01)**	0.31 (−3.06, 3.80)	0.648
n-PFOS	−3.85 (−9.09, 1.70)	0.47 (−3.02, 4.10)	−4.29 (−8.60, 0.22)*	0.658
Sm-PFOS	−3.02 (−7.18, 1.34)	−0.24 (−3.15, 2.77)	−1.70 (−5.54, 2.29)	0.765
Total PFOS	−4.09 (−9.24, 1.36)	0.42 (−3.07, 4.03)	−3.62 (−7.92, 0.87)	0.866
MeFOSAA	−2.86 (−7.31, 1.79)	3.14 (0.25, 6.12)**	−0.64 (−4.63, 3.52)	0.556
EtFOSAA	−2.26 (−5.73, 1.32)	2.70 (0.47, 4.98)**	−2.11 (−4.97, 0.85)	0.750
Testosterone				
n-PFOA	2.58 (−3.27, 8.79)	−0.13 (−4.21, 4.13)	−0.90 (−6.22, 4.72)	0.449
PFNA	3.18 (−3.07, 9.83)	2.82 (−1.18, 6.97)	0.67 (−4.17, 5.74)	0.520
PFHxS	1.47 (−3.23, 6.40)	1.09 (−1.83, 4.11)	0.14 (−3.77, 4.21)	0.722
n-PFOS	6.15 (−0.39, 13.12)*	1.31 (−2.68, 5.46)	−1.66 (−6.75, 3.70)	0.076
Sm-PFOS	1.30 (−3.64, 6.49)	1.25 (−2.12, 4.74)	−1.50 (−5.94, 3.15)	0.424
Total PFOS	5.24 (−1.15, 12.04)	1.34 (−2.65, 5.49)	−1.76 (−6.81, 3.55)	0.105
MeFOSAA	1.96 (−3.31, 7.51)	−2.86 (−5.98, 0.36)*	−1.46 (−6.04, 3.35)	0.364
EtFOSAA	0.13 (−3.91, 4.35)	−2.04 (−4.47, 0.46)	−1.63 (−4.92, 1.78)	0.551
SHBG				
n-PFOA	−1.11 (−6.84, 4.99)	−1.32 (−5.39, 2.92)	6.67 (0.91, 12.77)**	0.063
PFNA	0.21 (−5.98, 7.03)	−0.70 (−4.64, 3.41)	1.53 (−3.44, 6.76)	0.695
PFHxS	−0.10 (−4.84, 4.86)	−0.44 (−3.36, 2.57)	2.31 (−1.74, 6.52)	0.428
n-PFOS	−5.34 (−11.30, 1.01)*	0.90 (−3.12, 5.08)	5.82 (0.25, 11.69)**	0.010
Sm-PFOS	−3.68 (−8.44, 1.33)	−0.75 (−4.09, 2.72)	5.49 (0.68, 10.53)**	0.009
Total PFOS	−5.27 (−11.13, 0.98)*	0.45 (−3.55, 4.61)	6.42 (0.87, 12.27)**	0.006
MeFOSAA	−2.87 (−7.97, 2.51)	1.57 (−1.72, 4.98)	6.00 (1.04, 11.20)**	0.014
EtFOSAA	−3.57 (−7.54, 0.57)*	1.24 (−1.30, 3.84)	4.15 (0.66, 7.77)**	0.005

All models were adjusted for age (time-varying), race/ethnicity, site, smoking status, menopausal status (time-varying), and BMI (time-varying).
Abbreviations: E_2, estradiol; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; FSH, follicle-stimulating hormone; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; n-PFOA, linear perfluorooctanoate; n-PFOS, linear perfluorooctane sulfonate; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; SHBG, sex hormone-binding globulin; Sm-PFOS, sum of perfluoromethylheptane sulfonate isomers. *$P < 0.10$; **$P < 0.05$.

negative feedback regulators from granulosa cells, specifically E_2 and the inhibins (30). The declining number of follicles and decreasing follicular function with ovarian aging and the progression through the menopausal transition lead to decreased negative feedback and increased hypothalamic signaling to the pituitary gland, resulting in a progressive rise in FSH (31). The observed positive associations of PFOS and PFOA with FSH in the present study suggests that these compounds may accelerate ovarian aging through direct effect on ovarian negative feedback, potentially E_2 and the inhibins. This finding is in agreement with our previous report on the association between PFAS and earlier age of menopause (32). Only a few other studies have examined the association between serum PFAS concentrations and FSH, and the results have been mixed. A study of 120 Chinese women with overt POI and 120 healthy controls 20 to 40 years of age found that PFOS exposure was associated with lower E_2 and higher FSH levels in women with POI cases but not in controls (15). A study of 265 young female students 18 to 30 years

Table 4. Percentage changes (95% CIs) in serum concentrations of sex hormones for a doubling in PFAS concentrations that were selected in adaptive elastic-net models.

Selected PFAS in adaptive elastic-net	Percentage change (95% CI)
FSH	
n-PFOA	2.27 (0.26, 4.32)**
E_2	
n-PFOA	−1.01 (−2.50, 0.50)
PFNA	−1.10 (−2.48, 0.30)
MeFOSAA	1.09 (−0.08, 2.26)*
Testosterone	
n-PFOS	2.43 (−1.40, 6.42)
PFNA	1.70 (−1.50, 5.00)
MeFOSAA	−1.67 (−4.12, 0.84)
EtFOSAA	−1.88 (−3.92, 0.22)*
SHBG	
MeFOSAA	0.0001 (−2.22, 2.27)

All models were adjusted for age (time-varying), race/ethnicity, site, smoking status, parity, menopausal status (time-varying), and BMI (time-varying). *P <0.10; **P < 0.05.

Abbreviations: E_2, estradiol; EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; FSH, follicle-stimulating hormone; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; n-PFOA, linear perfluorooctanoate; n-PFOS, linear perfluorooctane sulfonate; PFAS, per- and polyfluoroalkyl substances; PFNA, perfluorononanoate; SHBG, sex hormone-binding globulin.

of age found no associations of PFOS and PFOA with FSH or with E_2, T, and SHBG (18). Accelerated ovarian aging attributable to PFAS exposure may not be observable in young, healthy women.

PFAS are structurally similar to fatty acids and have been identified as endocrine-disrupting chemicals (33). Endocrine disruption may be facilitated at the molecular level either by interaction of PFAS with the estrogen and/or androgen receptor or by an interference with sex hormone biosynthesis (11). Some PFAS possess weak estrogen-like effects; however, previous mechanistic studies suggest that these chemicals did not directly activate human estrogen receptor α, estrogen receptor β, or androgen receptor in vitro (34). It remains unclear whether PFAS affect estrogen receptor or androgen receptor pathways at concentrations relevant to human exposure. Nonetheless, PFAS could modulate the expression of estrogen-responsive genes, which are responsible for the maintenance of gonadotropin releasing hormone neurons in the hypothalamus (35,36). PFAS could also interfere with the negative feedback regulation of FSH by E_2 at the receptor level resulting in higher E_2 levels.

Our data also support a direct effect of PFNA and PFOA on E_2. PFAS have been shown to alter sex hormone steroidogenesis through activation of peroxisome proliferator-activated receptors (PPARs) and modulation of gene expression for enzymes responsible for cholesterol transport and ovarian steroidogenesis (37). Although human PPARs appear to be less responsive to PFAS than mouse PPARs, most perfluoroalkyl carboxylates and sulfonates activate PPARα and, to a lesser extent, PPARγ in mouse and human models (37). The ability to stimulate PPAR activation and further inhibit secretion of E_2 and possibly progesterone and androstenedione offers an alternative explanation, although weaker, nonsignificant associations between PFOS and E_2 compared to PFNA and PFOA in the present study are unclear. Toxicological studies have suggested that PFAS may disrupt ovarian steroidogenesis. In mice, PFOS administration for 4 to 6 months reduced the expression of genes responsible for transport of cholesterol as a necessary precursor for ovarian steroidogenesis (38). Epidemiologic studies also suggest a negative effect of PFAS on E_2. A Norwegian study reported a significant association of PFOS, but not PFOA, exposure with lower E_2 and lower progesterone levels in nulliparous, but not parous, women in a cross-sectional study of 178 healthy women 25 to 35 years of age (13). Similarly, Knox et al found lower E_2 was related to PFOS exposure but not PFOA among 25 957 women 18 to 65 years of age in the West Virginia C8 Health Project (12). In the Chinese POI study, PFOS exposure was associated with lower E_2 and higher FSH levels in women with POI cases but not in controls; however, no associations were detected for PFOA or with T (15).

We observed that the associations between PFAS and SHBG depended on parity. We tested effect modification by parity because it has been proposed that a valid test of ovarian toxicity of PFAS could be conducted in nulliparous women (13). Breastfeeding and reproductive characteristics associated with blood loss, including parity, menstrual bleeding, and menopause, have been associated with lower serum concentrations of PFAS (3,22,39,40) and may be important PFAS elimination pathways. With the same lifetime exposure to PFAS, nulliparous women would be expected to have higher body burden of PFAS compared with parous women, which may complicate evaluation of PFAS toxicity. SHBG is a glycoprotein binding globulin produced by the liver that transports sex steroids and determines the free fractions of both E_2 and T (41), with E_2 upregulating and T downregulating SHBG levels. While no evidence exists that PFAS directly suppress hepatic SHBG synthesis from animal models, it is possible that a PFAS-mediated reduction in E_2 signaling could explain the association.

The PFAS-SHBG association may operate through adiposity. In our study, a significant inverse association between PFOS and SHBG was observed when BMI was not in the model (Table 2). No significant effect modification by BMI suggests that the PFAS-SHBG association is independent of

adiposity. A potential link between PFAS and obesity has been reported (42,43), and in our population, baseline BMI was associated with serum PFAS concentrations. Hence, if past exposure to PFAS led to higher adiposity, which in turn influenced lower SHBG during the menopausal transition (44), the results with adjustment for BMI could be biased due to overadjustment. Toxicological studies are needed to elucidate underlying biological mechanisms.

The importance of evaluating of health effects of pollution mixtures has received considerable attention recently. However, statistical approaches for longitudinal data are limited, and no consensus exists regarding which approach is optimal (45). We, therefore, conducted an exploratory analysis and chose a 2-stage modeling approach to handle correlations in both dependent (linear mixed effects regression) and independent variables (adaptive elastic-net) (28). A major strength of elastic-net is its ability to identify important components (ie, variables with nonzero coefficients) in mixtures while handling the complex correlation structure of mixtures. In the present analysis, only n-PFOA remained statistically significant in relation to FSH, and beta coefficients for PFOS and its precursors were shrunk to zero. This shrinkage is due to modest to strong correlations between n-PFOA and PFOS and its precursors (MeFOSAA and EtFOSAA) [Spearman correlations, 0.41-0.82; see Supplemental Figure 2 in (21)]. This finding suggests that n-PFOA may be a factor that is causally associated with FSH while other PFAS may be proxies and their associations with FSH may be confounded by n-PFOA. However, it cannot be ruled out that n-PFOA was selected because its serum concentration captured true exposure more accurately than other compounds. An alternative approach is Bayesian kernel machine regression, which is designed to evaluate nonlinear associations as well as potential interactions in mixtures (46), but we were unable to implement it because of computational limitations.

The major strength of the present study was its longitudinal design. We were able to capture individual-level hormone concentrations over a 15-year period as women transitioned through the menopause. The multiracial/ethnic, community-based cohort permits the observed findings to be more generalizable than studies limited to 1 or 2 racial/ethnic groups or using clinic-based samples with medical conditions. The present study also had several limitations. First, we censored hormone data measured when women began hormone therapy. If women with high PFAS concentrations entered the menopausal transition earlier and were more likely to start hormone therapy, our findings may be biased toward the null. Second, PFAS were quantified only at baseline, 1999-2000. Around this time the population exposure might have peaked, especially for PFOS, because PFOS production started to phase out in 2000 (47), but since then, serum concentrations of PFOS and other PFAS have dropped (7,22,48). Despite long elimination half-life of several PFAS, including PFOS, exposure measurement bias cannot be ruled out. Third, we cannot rule out the possibility that the significant associations were observed by chance given the number of tests we conducted.

In conclusion, this longitudinal prospective study found positive associations of PFOA and PFOS with FSH and inverse associations of PFNA and PFOA with E_2 in midlife women during the menopausal transition, consistent with findings that PFAS affect reproductive aging. Parity may play a role in susceptibility to the PFAS-SHBG association. Given the widespread exposure in the general population, PFAS may be a potential risk factor for early ovarian aging and related chronic diseases later in life.

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